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RESEARCH ARTICLE

Assessment of 3-MCPD levels in coffee and coffee substitutes by simplified QuEChERS method

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Abstract A new simplified QuEChERS method, with the use of PSA sorbent and without the addition of magnesium sulphate, was established for the determination of 3-monochloropropane-1,2-diol (3-MCPD) in coffee and coffee substitutes. The final extracts were derivatised with phenylboronic acid and analysed by gas chromatography-mass spectrometry. The recoveries were in the ranges of 90.5–107 % for all coffee types. The calculated limit of detection was 2.5 μg kg^{-1} and the linearity within the range of 2–200 ng mL^{-1}. In real samples the highest level of 3-MCPD was detected in soluble coffee surrogates (average 135.6 μg kg^{-1}), especially in those prepared from roasted spelt or barley, where they reached even up to 398.1 μg kg^{-1}. The contents of 3-MCPD in natural instant and ground coffee samples were much lower with the average of 16.6 and 14.1 μg kg^{-1}, respectively. Any impact of factors such as manufacturer or coffee origin was not observed.

Keywords Coffee · Coffee substitute · 3-Monochloropropane-1,2-diol, 3-MCPD · QuEChERS, GC–MS

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1 Introduction

Coffee and coffee substitutes (surrogates), prepared from roasted cereals or vegetables, are some of the most popular beverages in the world. Roasting, a crucial step for its production, allows developing colour, aroma and flavour, which are essential and specific for coffee and coffee substitutes (Belitz et al. 2009). However, roasting may lead to the formation of undesirable compounds, such as chloropropanols. Chloropropanols were first recognized in acid-hydrolyzed vegetable protein (HVP), which is a flavouring agent and is used in soy sauce production. The most abundant chloropropanol found in foodstuff is 3-monochloropropane-1,2-diol (3-MCPD). Apart from acid hydrolysis, 3-MCPD appears to form from lipids and sodium chloride during thermal processing of food with a low water content at the temperature above 160 °C (Chung et al. 2008). 3-MCPD can be also released during degradation of 3-MCPD esters—the bound form with higher fatty acids—or by migration from certain types of epichlorohydrin-based wet strength resins used in paper and cellulose casings (Wenzl et al. 2007; Chung et al. 2008). In natural coffee 3-MCPD is formed during the roasting process from salt and lipids naturally present in coffee beans. In grain-derived products, such as coffee substitutes, 3-MCPD results from the dry-kilning of barley at temperatures above 170 °C (Baer et al. 2010). The 3-MCPD was detected in coffee and coffee surrogates at levels up to 759 μg kg^{-1} (Kurzrock and Speer 2007). 3-MCPD was classified in 1995 as genotoxic carcinogen by The European Commission's Scientific Committee for Food and a provisional maximum tolerable daily

intake of 2 μg kg^{-1} body weight has been established in EU (European Commission 2001). According to the Regulation 1881/2006 maximum levels of 3-MCPD at 20 μg kg^{-1} were set only for HVP and soy sauce (Commission Regulation 1881/2006).

The conventional sample preparation method for 3-MCPD consists of extraction with various ethers, ethyl acetate, dichloromethane, or mixture acetone with hexane. Clean-up of the extract is conducted mainly with a column chromatography loaded with alumina, diatomaceous earth or an Extrelut column, followed by derivatisation mostly with PBA (phenylboronic acid), heptafluorobutyrylimidazole (HFBI) or heptafluorobutyric acid anhydride (HFBA) and with a final detection by gas chromatography (Rétho and Blanchard 2005; Baer et al. 2010; Racamonde et al. 2011). Many of these procedures do not perform well in more complex and difficult matrixes such as coffee or coffee substitutes, due to the presence of undesirable co-extractives that produce interferences affecting chromatographic analysis. An alternative is the use of the QuEChERS approach, based on extraction with acetonitrile followed by a dispersive solid-phase extraction (dSPE) and analysis by gas or liquid chromatography-mass spectrometry. The method have been extensively applied for various food and environmental contaminants such as pesticides, drugs, veterinary medicines, mycotoxins, chloroalkanes, phenols, perfluoroalkyl substances and PAHs (Bruzzoniti et al. 2014). However, so far, to the best of our knowledge, there has been no attempt to adapt the QuEChERS method for the determination of 3-MCPD in food samples. Thus, the aim of this study was to evaluate the use of a simplified variant of the QuEChERS method for the assessment of 3-MCPD levels in coffee and coffee substitutes.

2 Materials and methods

2.1 Chemicals and reagents

Hexane, ethyl acetate, acetone and acetonitrile and HPLC grade for liquid chromatography LiChrosolv® were purchased from Merck KGaA, Germany. Sodium chloride and magnesium sulphate p.a, was purchased from Chempur SA, Poland. primary secondary amine (PSA), strong anion exchange (SAX), and florisil SPE Bulk Sorbent derived from Agilent Technologies, USA. Celite® 545, 3-monochloropropane-1,2-diol (3-MCPD), 3-monochloropropane-1,2-diol-d_5 (3-MCPD-d_5) (internal standard), 3-monobromochloropropane-1,2-diol (3-MBPD) (syringe standard), and phenylboronic acid (PBA) (derivatisation agent) were obtained from Sigma-Aldrich, USA. Stock, intermediate and working standard solutions of chloropropanols at a concentration of 2 μg mL^{-1} were prepared in ethyl acetate. PBA solution was prepared by dissolving 5 g PBA in 20 mL mixture of acetone and water (19:1, v/v). A sodium chloride solution of 200 mg mL^{-1} (20 %) was prepared in deionized water.

2.2 Instrumentation

The Varian 4000 GC/MS (Varian, Inc., USA) system consisting of a 3800 GC with a CP-8410 auto-injector (Bruker, USA) and a 4000 Ion Trap MS detector was used to perform the analyses. The injector was a CP-1177 Split/Splitless Capillary Injector, with a temperature of 250 °C, and an injection volume of 1.0 μL. Each injection was performed in triplicate. Chromatographic separations were conducted using a DB-5MS column (30 m × 0.25 mm × 0.25 μm; Agilent Technologies, USA). The GC oven was operated with the following temperature program: initial temperature 50 °C (1.0 min), 10 °C min^{-1}, 210 °C (1.0 min), 30 °C min^{-1} and 250 °C (5.0 min). The analyses were carried out with a solvent delay of 8.0 min. Helium 5.0 (Linde Gas, Poland) was used as the GC carrier gas at a flow rate of 1.0 mL min^{-1}. The ion trap mass spectrometer was operated in the internal ionisation mode, scan from *m*/*z* 45–500. The emission current of the ionisation filament was set at 15 μA. The trap and the transfer line temperatures were set at 180 and 220 °C, respectively. Analyses were conducted in the selected ion monitoring mode (SIM) based on the use of one quantitative ion of PBA derivatives (147 for 3-MCPD and 3-MBPD, 150 for 3-MCPD-d_5), qualitative ions (196 for 3-MCPD, 201 for 3-MCPD-d_5 and 241 for 3-MBPD) and retention times (13.40, 13.45 and 14.57 for 3-MCPD-d_5, 3-MCPD and 3-MBPD, respectively). Acquisition and processing data were performed using Varian Start Workstation software and NIST 2.0 library. MS1 Minishaker (IKA, Germany), MPW 350 R Centrifuge (MPW Med. Instruments, Poland) were employed during the sample preparation. AccublockTM (Labnet, USA) with nitrogen 5.0 (Linde Gas, Poland) was used to evaporate the solvent, and concentrate the extracts.

2.3 Sample preparation: optimisation experiment

The recovery experiment was separately undertaken for natural instant coffee, natural ground coffee and coffee substitute. 1 g of the sample was weighted into

Table 1 Amounts of sorbents used in tested combinations

Tested sorbent combination	Amount of sorbents
I	300 mg florisil
II	300 mg PSA
III	300 mg celite
IV	500 mg PSA
V	150 mg PSA + 300 mg florisil + 300 mg celite
VI	150 mg PSA + 150 mg SAX
VII	150 mg PSA + 300 mg florisil
VIII	150 mg PSA + 300 mg celite

PSA primary secondary amine, *SAX* strong anion exchange

a 50 mL centrifuge tube, spiked with 3-MCPD and 3-MCPD-d_5 solutions to the level of 50 μg kg^{-1}, mixed and left to stand for 15 min at room temperature prior to extraction. The best variant of the sorbent combination was tested also at the fortification level of 5 μg kg^{-1}. Then, 10 mL of hot, boiled water was added to the sample. After cooling to a room temperature, 10 mL of acetonitrile was added and the mixture was shaken vigorously for 1 min. Next, 1 g of NaCl was added, the sample was shaken vigorously for 1 min, and centrifuged for 15 min at 9000 rpm. 6 mL of the supernatant was transferred into a PP 15 mL tube containing appropriate amount of tested sorbents (Table 1). The tube was shaken for 2 min and centrifuged for 15 min at 10,000 rpm. A 4 mL amount from the extract was transferred into a screw cup vial and the extract was evaporated under a stream of N_2 to dryness. The residues were dissolved in 100 μL of 20 % NaCl aqueous solution and 50 μL of 3-MBPD solution and 50 μL PBA solution were added. The mixture was heated at 90 °C for 20 min. After cooling 0.5 mL of hexane was added, the mixture was shaken vigorously and 200 μL of upper hexane layer was transferred into an insert in an autosampler vial. The extracts were then analysed by GC–MS. Blank samples and reagent blanks were prepared similarly but were not fortified. Each sample was prepared in triplicate.

2.4 Standard preparation

A series of standard solutions in ethyl acetate were prepared by dilution of the standard mixture solution at the following concentrations: 2, 5, 10, 40, 70, 100 and 200 ng mL^{-1}. An appropriate volume of standard solution was evaporated to dryness, and then treated in a similar way to the samples. The GC–MS

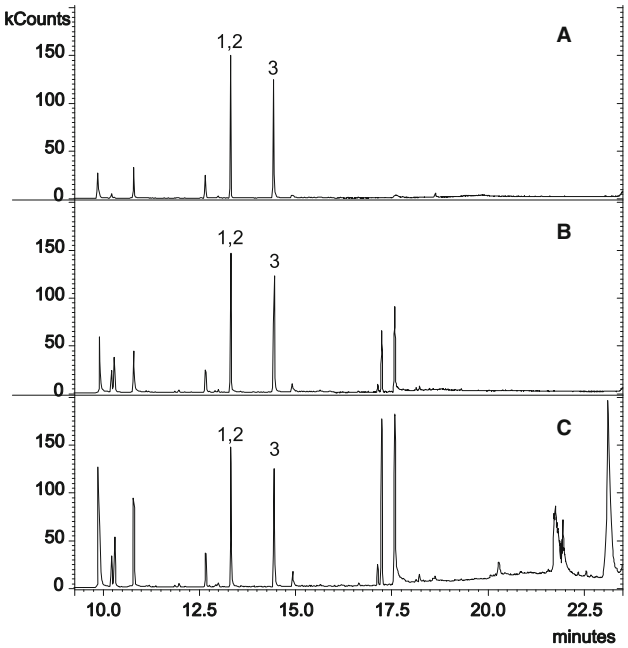

Fig. 1 GC-MS chromatograms of standards solution at the concentration of 40 ng mL^{-1} (**a**), fortified sample (50 μg kg^{-1}) cleaned-up with 300 mg PSA (**b**), fortified sample (50 μg kg^{-1}) cleaned-up with 300 mg celite (**c**); *1*, 3-MCPD-d_5, *2*, 3-MCPD, *3*, 3-MBPD

chromatogram of standards at the concentration of 40 ng mL^{-1} was displayed in Fig. 1 (chromatogram A).

3 Results and discussion

3.1 Optimization of sample preparation method

Taking into account the presence of interfering matrix components of coffee (Sadowska-Rociek et al. 2015) PSA and SAX sorbent were chosen to test the clean-up of the extracts. Other filter agents and sorbents, such as Celite (diatomaceous earth) and florisil, used in previously published studies (Rétho and Blanchard 2005; Racamonde et al. 2011), were also tested in our experiment. However, in our experiment all materials were used as bulk sorbents, being added to the extracts. The best recoveries, within 75–110 % (Commission Regulation 836/2011) were observed in all combinations (I–VIII) for instant coffee, I, II, III and VI for coffee substitutes and I, II and III for ground coffee (Fig. 2). Combinations II and III, for which the recoveries were acceptable for all types of coffee, included the use of 300 mg PSA (II) or 300 mg Celite (III). The final selection of an appropriate sorbent was based on the presence of the interfering compounds at the chromatograms (Fig. 1). The application of 300 mg PSA

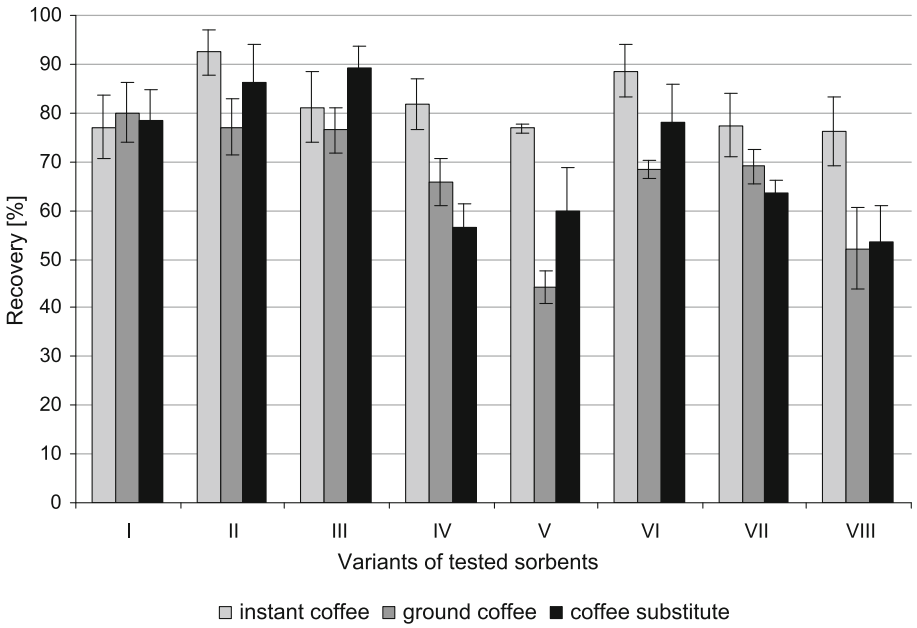

Fig. 2 Recovery values for combinations of sorbents tested

(chromatogram B), in opposite to Celite (chromatogram C), showed the best results in removing of the interferents, and resulting in acceptable recovery values (92.5, 86.1 and 77.1 % for instant coffee, coffee substitute and ground coffee, respectively). The recovery experiment with PSA sorbent was repeated at the fortification level of 5 μg kg^{-1} giving the recovery values 81 % for instant coffee, 109 % for coffee substitute and 76 % for ground coffee. Hence, PSA sorbent was selected for further experiments.

The innovation in this work was the use of the QuEChERS approach for sample preparation. The original QuEChERS method is based on analyte extraction with the use of acetonitrile, addition of sodium chloride to separate water and organic phase, and magnesium sulfate for the removal of residual water. Organic phase is then cleaned-up by dispersive SPE employing bulk sorbents and $MgSO_4$ once again (QuEChERS 2015). The application of $MgSO_4$ is necessary to remove any trace of water, especially in case of GC–MS analysis. However, in our experiment $MgSO_4$ was not employed, because it had been assumed that the final extract would have been re-dissolved in aqueous NaCl solution, so the removal of water prior to derivatisation would be useless. This simplified QuEChERS method involved only the acetonitrile extraction, addition of NaCl to separate the organic layer, and bulk sorbent addition. In order to compare the recovery of the simplified method, an original QuEChERS method (with $MgSO_4$) was also applied. The observed recoveries at the spiking level of 50 μg kg^{-1} were slightly higher (95, 88 and 78 % for instant coffee, coffee substitute and ground coffee). Despite these higher recoveries, a simplified version of the QuEChERS method was preferred, because of its quickness and simplicity.

The repeatability of the optimized method, expressed as the relative standard deviation (RSD) of the spiked sample concentrations, was lower than 9 % for all coffee types at both spiking levels. The linearity in the range 2–200 ng mL^{-1}, expressed as the correlation coefficient of the calibration slope, was higher than 0.99. Limits of detection (LOD) were estimated as three times higher than the level of noise measured at GC–MS chromatogram at the lowest calibration level. The limit of quantification (LOQ) was equal to three times higher than LOD. The values obtained were: 2.5 and 7.5 μg kg^{-1} for LOD and LOQ, respectively.

In summary, the simplified QuEChERS method proved to be simpler, more rapid and, above all, more cost effective and user friendly compared to classical sample preparation treatments. The acetonitrile extraction allowed avoiding the use of toxic and hazardous extraction solvents, such as ethers or organochlorines. Only small amounts of sample (1 g) and reagents (up to 10 mL) were needed. What is more, the addition of magnesium sulfate (about 5 g/sample) was also omitted which resulted in little waste generating. The application of bulk sorbents permitted to eliminate the need for clean-up of laboratory equipment used (glass columns, SPE systems, etc.). The proposed approach excluded also some laborious multistage procedures such as liquid–liquid extraction in a separatory funnel, shortening the extraction time to 1 min, while classical methods

require between 10 and 20 min (Carro et al. 2013). Besides the above mentioned advantages of the method, it showed good results in the removal of co-extracts and acceptable recoveries. In case of coffee matrices, the proposed concept led to similar recoveries (~99 %) but lower LOD than reported by Doležal et al. (2005) (2.5 and 3 μg kg^{-1}, respectively).

3.2 Real sample analysis

In the second part of the study 30 samples of coffee (10 samples of natural ground coffee, 10 samples of natural instant coffee, and 10 samples of coffee substitutes) were subjected to 3-MCPD determination using previously optimized procedure. All samples were collected from the Polish market in 2014. The highest level of 3-MCPD (average of 10 samples: 135.6 μg kg^{-1}) was observed for coffee substitutes, while for natural ground and instant coffee the average 3-MCPD levels were much lower (16.6 and 14.1 μg kg^{-1}; Table 2). The results for the samples of natural coffee, both ground and instant were in the ranges 6.2–29.0 and 4.6–33.5 μg kg^{-1}. Although some of the samples were delivered from the same manufacturer, any impact of the coffee origin on 3-MCPD content were not found. No influence of coffee colour or coffee cultivar (Arabica or Robusta) on the level of 3-MCPD was observed. Within the coffee substitutes the samples of soluble coffee substitutes (1-6CS) were significantly more contaminated with 3-MCPD than the samples of insoluble coffee substitutes (7-10CS). The only exception was the sample 6CS, in which the level of 3-MCPD was not as high as in other instant coffee substitutes. This phenomenon probably might result from the origin and composition of these samples. Almost all coffee substitutes constituted a mixture of roasted barley and/or rye and vegetables such as sugar beet and chicory, whereas the sample 6CS was prepared only from roasted chicory. On the other hand, the sample 5CS with the highest level of 3-MCPD (398.1 μg kg^{-1}) was produced only from roasted spelt without any other components. Finally, within the coffee surrogates containing the mixture of roasted materials (samples 1–4 and 7–9CS), the lowest level of 3-MCPD was found in the samples without roasted barley (7-9CS). The difference in coffee composition and its influence of the results of 3-MCPD levels confirm that roasted cereals, mainly spelt and barley, especially in instant form, are the main source of this contaminant in coffee substitutes.

Maximum tolerable daily intake of 3-MCPD has been established to 2 μg kg^{-1} body weight, so for a mean body weight of 70 kg the intake should be not higher than 140 μg. Assuming that the average amount of coffee consumed is 6 teaspoons per day (3 cups), the daily dose of the compound is only about 12 μg (calculated for sample 5CS with the highest detected level of 3-MCPD).

Our findings are comparable to those from other studies. Doležal et al. (2005) reported 3-MCPD levels in ground coffees at the ranges of 10.1–18.5 μg kg^{-1}. The highest 3-MCPD level was found in one instant coffee sample and in the coffees with very long time application during roasting. The 3-MCPD contents in samples of natural coffee from the German market (Kurzrock and Speer 2007) were lower than 20 μg kg^{-1} while for coffee surrogates varied in the ranges 43–759 μg kg^{-1} and the lowest amounts of 3-MCPD were determined in the insoluble samples

Table 2 3-MCPD level in coffee samples analysed

3-MCPD content (μg kg^{-1})					
Coffee substitutes		Instant coffee		Ground coffee	
1CS[a]	153.7 ± 8.6	1IC	17.8 ± 0.6	1RC	8.1 ± 1.1
2CS[a]	119.8 ± 2.4	2IC	<LOQ (6.2)	2RC	13.2 ± 0.3
3CS[a]	158.5 ± 1.1	3IC	25.8 ± 0.7	3RC	<LOQ (6.1)
4CS[a]	251.8 ± 20.3	4IC	29.0 ± 1.0	4RC	33.5 ± 1.4
5CS[a]	398.1 ± 4.9	5IC	27.9 ± 1.1	5RC	20.5 ± 1.2
6CS[a]	43.9 ± 2.0	6IC	<LOQ (6.2)	6RC	13.2 ± 0.8
7CS	60.8 ± 3.2	7IC	8.0 ± 2.1	7RC	9.0 ± 1.3
8CS	43.7 ± 3.2	8IC	12.4 ± 0.7	8RC	<LOQ (4.6)
9CS	27.8 ± 1.2	9IC	14.9 ± 0.4	9RC	23.2 ± 0.7
10CS	97.8 ± 8.0	10IC	18.1 ± 0.9	10RC	9.9 ± 1.6

CS coffee substitute, *IC* natural instant coffee, *RC* natural ground coffee

[a] Instant (soluble) coffee substitute

(43–120 μg kg^{-1}). In the study provided by Divinová and Velíšek (2007) the insoluble coffee surrogates had the free 3-MCPD level in the range of <9.0–32 μg kg^{-1}. The highest amount was observed in one sample of roasted barley whereas the lowest level in roasted rye.

3.3 Conclusions

A simplified QuEChERS method for the determination of 3-MCPD in coffee surrogates and natural coffee samples has been proposed. The usefulness of the method has been confirmed in terms of quickness, simplicity, reduction in waste and analytical performance including method recovery values, precision, LOQ and linearity. In the monitoring study, the highest level of 3-MCPD was detected in the soluble coffee substitutes, especially in those prepared with roasted barley. The contents of 3-MCPD in both ground and instant natural coffee were lower and any impact of factors such as manufacturer or coffee origin was not observed.

Conflict of interest The authors declare that they have no conflict of interest.

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